2098-Pos Board B84
A Hydrodynamic Analysis of the Human Cullin 5 - hIV-1 Vif Ubiquitin Ligase Complex
Stephen Techtmann, Rodolfo Ghirlando, Ernest Maynard.
The HIV accessory protein virion infectivity factor (Vif) is essential for viral replication in CD4+ cells. In the absence of Vif, HIV is efficiently restricted by the cellular cytidine deaminase, APOBEC3G (A3G). Vif recruits a cullin 5 (Cul5)-based ubiquitin ligase and Elongin B/C, which target A3G for proteasomal destruction. Many cullin-based ubiquitin ligases have been shown to oligomerize via their substrate binding motifs. It has also been reported that Vif oligomerizes, but the nature of these oligomers is unknown. We have employed biochemical and biophysical techniques to investigate the oligomeric properties of Vif and Cul5 alone and in complex.
Using analytical ultracentrifugation (AUC) we have characterized Vif, Cul5, and Elongin B/C and their complexes. This work will allow for an examination of how the oligomeric state of the free proteins effects the Vif-Cul5 interaction. From these studies we determined that Cul5 dimerizes with a Kd of 70 μM. Our initial studies with Vif (101-153) show little oligomerization. AUC analysis of the Elongin B/C shows that the predominant species is the heterodimer and some higher order oligomerization is observed. We have analyzed the Vif-Cul5-Elongin B/C complex and have seen that the stoichiometry of the complex is 1:1:1:1. Therefore it appears that the oligomeric forms of the individual proteins are not maintained in the complex. It is possible that the sites of self-association occur at the interfaces between the binding partners. To further characterize the Vif-Cul5-Elongin B/C interaction, isothermal titration calorimetry (ITC) was used to define the thermodynamic properties governing association. A thorough understanding of the mechanism of the Vif-Cul5-Elongin B/C complex will shed light on the possible role of oligomerization in both ubiquitin ligase function as well as the function of HIV-1 Vif.

2099-Pos Board B85
Expression and Purification of the BK Channel Alpha-Beta1 Complex
Yixuan Zhang, Yangyang Yan, Youshan Yang, Fred Sigworth.
Large conductance Ca2+ activated potassium channels (BK) are composed of pore-forming alpha-subunits and can be associated with modulatory beta-subunits. The beta1 subunit is mainly expressed in smooth muscle, and is found to increase the Ca2+ sensitivity of BK channels and slow the deactivation process. We have generated HEK293 cell lines stably expressing the hSlo alpha subunit and beta1 subunit genes. The hSlo construct carries N-terminal Flag and His tags for purification, while the mouse beta1 is fused with GFP at the C-terminus. Current recordings from inside-out patches showed a shift in the voltage sensitivity of activation consistent with co-assembly of the subunits. The alpha-beta1 complex was bound in the presence of decylmaltoside to Flag antibody affinity beads, and eluted with Flag peptide. Co-expression was verified by protein gels and western blots, and quantitative measurement of GFP fluorescence allowed the stoichiometry of the complex to be estimated.

2100-Pos Board B86
Assembly and Function of Pore-Forming Toxin Aerolysin from Aeromonas Hydrophila
Matteo Dal Peraro, Matteo Degiacomi, Ioan Iacovache, Gisou van der Goot.
One of the most ancient forms of attack between cells or organisms has been the production of proteins or peptides that affect the permeability of the target cell membrane. This class of weapons includes the largest family of bacterial toxins, the pore-forming toxins (PFTs). PFTs are bistable structures that can exist in a soluble and a transmembrane state. It is unclear what drives folding towards both the monomeric soluble state (a requirement that is essential to protect the PFT producing cell), and the final functional form, which is a heptameric transmembrane spanning pore on the attacked cell. We have investigated folding and the assembly of aerolysin, produced by the human pathogen Aeromonas hydrophila and more specifically the role of the C-terminal propeptide (CTP) and the pore-forming loop for the folding of the soluble and functional pore state. By combining the predictive power of computational techniques (e.g. molecular dynamics simulations) with experimental validation using both structural and functional approaches, we show that the CTP is essential for folding. Aerolysin CTP is crucial in the control of toxic activity since it catalyzes folding of the individual subunits within the bacterium and later controls assembly of the quaternary pore-forming complex at the surface of the target host cell. Based on these data and on the complete characterization of aerolysin domains flexibility we were able to obtain the intermediate pore structure using a newly developed method able to reconstruct the assembly from the structure of the single subunit and low-resolution cryo-EM maps of the final oligomer. These results validated by site directed mutagenesis allowed us to build an accurate structural model of the final functional pore of aerolysin.

2101-Pos Board B87
Viscoelasticity of Hemoglobin S Gels: Moduli, Kinetics, Structure and Mechanisms
Robin W. Briehl, Suzanna Kwong.
Red cell rigidification and damage due to polymerization and gelation of deoxygenated sickle cell hemoglobin lie at the root of pathogenesis in sickle cell disease. Although the abnormal blood rheology and gel viscosity have been studied, viscoelasticity has been little examined. Using sensitive coneplate dynamic rheometry at low shear, we followed development of gels kinetically over 5 decades of increasing elasticity, G' and loss modulus, G", until final levels were reached. Viscoelasticity progresses through 3 regimes. (1) A rapid, brief increase in G' and G" that occurs after initial nucleation can be explained as an initial quadratic dependence on time predicted to result from linear progress of both initiating nucleation and fiber growth with time. The percolation transition from fluid to solid viscoelasticity may also contribute to early rheological change. (2) Following stage (1), G' and G" increase exponentially, consistent with the dominance of heterogeneous nucleation of new fibers on the existing fiber mass. Exponential rates, B=dlnG/dt, scale as the approximate 100th power of solution hemoglobin concentration. The increasing viscoelasticity depends on polymer density, known to increase exponentially, but is also affected by two patterns of interfiber cross-linking and by domain size, packing, overlap and interdigitation. (3) Viscoelasticity reaches asymptotic levels. Final levels of G' and G" have a low, between linear and quadratic, power dependence on gel density. At 15 mM(heme), about 3/4 the normal red cell concentration, G'.150 KPa. This three stage sequence shows more complexity for concentrations near 14 mM heme, with changes in exponential rate during stage (2): slow exponential progress is followed by reacceleration. The slow, near-plateau, regime may depend on entanglements in a rubber-like polymeric system, but with the addition of continuing polymerization that causes residual increase in the plateau modulus, G_e.

2102-Pos Board B88
Minimalistic Approach to Protein Assembly Modelling/Application to the Sickle Cell Hemoglobin Polymerization
Bogdan Barz, Christopher Kepics, Frank A. Ferrone, Brigita Urbanc.
Aberrant assembly of proteins into oligomers and fibrils is associated with many diseases such as Alzheimer's disease, Parkinson's disease, Huntington disease, amyotrophic lateral sclerosis, type II diabetes, systemic amyloidosis, sickle cell anemia, etc. Due to limitations of fully atomistic computational approaches, coarse-grained models provided important insights into protein assembly in general as well as in particular as in the case of amyloid beta-protein, for which discrete molecular dynamics (DMD) using a four-bead protein model with implicit solvent successfully elucidated oligomer formation. Here we addressed the question of how to construct a minimal model of protein assembly using the DMD computational method. We found that a tetrahedron model of a monomer with both effective hydrophobic and hydrophilic interactions accounts for a description of an entire pathway of assembly from monomeric, through transient oligomeric to elongated ordered assemblies.
A more specific and challenging problem is the model for the sickle cell hemoglobin polymerization. A genetic mutation (E6V) on the two beta chains of the hemoglobin tetramer allows effective hydrophobic interactions at these sites with other hydrophobic sites. As a result, the sickle cell hemoglobin polymerizes into long fibers that eventually change the shape of the red blood cell. There are experimentally identified lateral and axial contacts on the mutated hemoglobin that contribute to assembly into a 14-strand fiber structure. Currently there are no computational models to predict the assembly of sickle cell hemoglobin into helical fibers. We have developed a 9-bead DMD model for the sickle cell hemoglobin, based on the crystal-structure sites known to be involved in the assembly. The relative location of the axial and lateral contacts determines the structure of the final fiber as well as intermediate structures observed on the assembly pathway.

2103-Pos Board B89
Nano-Scaled Three-Dimensional Fibrillar Network Made of Curly Amyloid Fibrils of α-Synuclein
Ghibom Bhak, Chul-Seok Hong, Seung R. Paik.
Amyloid fibrils are highly organized protein suprastructures derived from soluble peptides and proteins through the specific self-assembly process. From a single amyloidogenic protein of α-synuclein, two distinctive amyloid fibrils

were produced depending on the fibrillation processes. The polymorphism resulting in formation of curly (CAF) and straight amyloid fibrils (SAF) was respectively achieved with the centrifugal membrane filtration of the preformed α-synuclein oligomers and the agitated incubation of its monomeric form. It is demonstrated that the production of CAF and SAF represents two parallel mechanisms of amyloidogenesis via double-concerted and nucleation-dependent fibrillation process, respectively. Differences in their secondary structures of the polymorphs have been suggested to be responsible for their characteristic morphologies with significant variations in physical properties, which were inherited for two consecutive generations to the daughter and granddaughter fibrils by self-propagation property. Accumulation of highly flexible and mechanically strong CAF eventually produced the hydrogel composed of the three-dimensional fibrillar network in fine nano-scale. The amyloid hydrogel was proven to be a suitable nanomatrix for enzyme entrapment, protecting the immobilized enzyme from the activity decrease due to repetitive catalytic reactions and heat treatment. Therefore, the nano-scaled fibrillar network of CAF is expected to be employed for various future applications in nanobiotechnology including drug delivery, tissue engineering and biosensor development.

2104-Pos Board B90

Isolating Toxic Insulin Amyloid Oligomers that Lack Beta-Sheets and have Wide pH Stability

Caryn L. Heldt, Dmitry Kurouski, Mirco Sorci, Elizabeth Grafeld, Igor K. Lednev, Georges Belfort.

Amyloid diseases, including Alzheimer's and Parkinson's disease, are characterized by aggregation of normally functioning proteins or peptides into ordered, beta-sheet rich fibrils. There are many theories on the species that causes toxicity in amyloid diseases, mainly focused on the nuclei or oligomers in the fibril formation process. The nuclei and oligomers are transient species, which makes their full characterization difficult. We have isolated a toxic protein species that acts like an oligomer and may provide evidence of a stable oligomer. This oligomer was isolated by dissolving amyloid fibrils at high pH, then purified and concentrated by diafiltration. It has a mass greater than 100 kDa and a diameter ranging of 48 $\pm$ 15 nm. The oligomer seeds the formation of fibrils in a dose dependent manner and exhibits Thioflavin-T fluorescence, but circular dichroism and deep UV resonance Raman spectroscopy did not find any evidence of an increase in beta-sheet structure. It appears that this oligomer is largely unstructured protein. We hypothesize that the oligomer does not decompose at high pH and maintains its structure in solution. All of the insulin, however, that joined the oligomer to elongate the beta-sheet rich fibrils folded in a different conformation and could be removed from the fibril and returned to native, dissolved insulin. This is the first time that a stable oligomer of an amyloid reaction has been separated and characterized without genetically engineering the protein or having additives in the fibrillation media. It appears that the oligomer does not have the same structure as the fibrils and is possibly intrinsically unfolded. This may make the search for a stable amyloid oligomer or nucleus even more difficult since methods to detect beta-sheets are often employed in the search for these structures.

2105-Pos Board B91

Structural Basis for Amyloid β-Protein Toxicity Inhibition: A Multiscale Computational Study

Brigita Urbanc, Bogdan Barz, Mark Betnel, Luis Cruz, Gal Bitan, David B. Teplow.

Amyloid β-protein (Aβ) oligomers play a central role in Alzheimer's disease. Of two predominant Aβ alloforms, Aβ40 and Aβ42, Aβ42 is more toxic. *In vitro* Aβ40 and Aβ42 oligomerize through distinct pathways. Here we demonstrate that discrete molecular dynamics (DMD) with a coarse-grained protein model captures experimentally observed oligomerization differences between Aβ40 and Aβ42 and provides experimentally unattainable structural information, critical to understanding the structural basis of Aβ42 toxicity. Our results show that DMD-derived Aβ40 and Aβ42 oligomer structures differ significantly at the N-terminal regions. The region A2-F4 is involved in Aβ40, but not in Aβ42, oligomerization, resulting in an increased solvent exposure of the N-terminal region in Aβ42 relative to Aβ40 oligomers. Selected DMD-derived Aβ40 and Aβ42 dimer conformers were further assessed for stability using all-atom MD in explicit solvent. Consistent with the DMD conformers, this analysis shows that in Aβ42 dimers, the N-terminal region is significantly more disordered and exposed to solvent than in Aβ40 dimers. The DMD approach was then applied to study Aβ42 oligomerization in the presence of three Aβ-derived C-terminal fragments (CTFs), which were previously shown to inhibit Aβ42 toxicity in cell cultures, and a control Aβ fragment, which had no effect on toxicity. We show that CTFs co-assemble with Aβ42 to form large Aβ42/CTF heterooligomers while the control fragment promotes monomeric states and formation of small heterooligomers. The presence of CTFs reduces the average β-strand structure in Aβ42 and increases the solvent exposure of the D1-D7 region of Aβ42 while the control peptide has the opposite effect on both quantities. Based on these findings, we hypothesize that the increased β-strand propensity and/or the increased solvent exposure of the D1-D7 region in Aβ42 relative to Aβ40 oligomers are key structural elements mediating Aβ42 toxicity.

Molecular Chaperones

2106-Pos Board B92

Elucidating the Mechanism of Protein Remodeling by the Hsp90 Molecular Chaperone

Timothy O. Street, Laura Lavery, David A. Agard.

Hsp90 is a ubiquitous molecular chaperone that interacts with highly diverse protein substrates. Previous structural analysis demonstrated that Hsp90 can adopt a large number of structurally distinct conformations, however the functional role of this flexibility is not understood. We investigate the structural consequences of substrate binding with a model system in which Hsp90 interacts with a constitutively unfolded protein (Δ131Δ), a well-studied fragment of staphylococcal nuclease. SAXS measurements reveal that under apo conditions Hsp90 partially closes around Δ131Δ and in the presence of AMPPNP Δ131Δ binds with increased affinity to Hsp90's fully closed state. Δ131Δ accelerates the nucleotide-driven open/closed transition and stimulates ATP hydrolysis by Hsp90. NMR measurements reveal that Hsp90 binds to a specific region of Δ131Δ. Although Δ131Δ is globally unfolded this particular region is significantly structured. These results indicate that Hsp90 can bind a locally structured region in a globally unfolded protein and this binding drives conformational and functional changes in the chaperone.

2107-Pos Board B93

Direct Observation of Torsional Motion of Group II Chaperonin

Hiroshi Sekiguchi, Ayumi Nakagawa, Kazuki Moriya, Kouhei Ichiyanagi, Shin-ichi Adachi, Masafumi Yohda, Yuji C. Sasaki.

Diffracted X-ray Tracking (DXT) has been considered as a powerful technique in biological science for detecting subtle (pico meter scale) dynamic motion of the target protein at single molecular level. This method was applied for various proteins, such as bacteriorhodopsin [1], antibody [2] and KcsA channel [3]. In DXT, the dynamics of a single protein can be monitored through trajectory of the Laue spot from the nanocrystal which was labeled on the objective protein immobilized on the substrate surface.

In this study, DXT method was applied to the group II chaperonin, a protein machinery that captures an unfolded protein and refolds it to the correct conformation in an ATP dependent manner [4]. A mutant group II chaperonin from Thermococcus strain KS-1 with a Cys residue at the tip of the helical protrusion, was immobilized on the gold coated substrate surface and was labeled with a gold nanocrystal through gold-thiol bond.

We monitored diffracted spots from the nanocrystal as dynamic motion of the chaperonin, and found that the rotational motion of the nanocrystal, which corresponded to the torsional motion of the chaperonin, in the presence of ATP condition was 10 times larger than that in the absence of ATP condition. And UV-light triggered DXT study using caged ATP revealed that the chaperonin twisted counterclockwisely (from the top to the bottom view of chaperonin) when ATP binded to the chaperonin and the angular velocity from open to closed state of chaperonin chamber was 10 % faster than that from closed to open state.

[1] Y. Okumura et al., Phys. Rev. E, 70:021917 (2004)
[2] T. Sagawa et al., Biochem. Biophys. Res. Commun. 335:770 (2007)
[3] H. Shimizu et al., Cell 132:67 (2008)
[4] T. Kanzaki et al., J. Biol. Chem. 283: 34773 (2008)

2108-Pos Board B94

Structural and Functional Studies of the *E. Coli* ClpA Molecular Motor

Aaron L. Lucius.

ATP dependent proteases, such as the *E. coli* ClpAP and eukaryotic 26 S proteasome are critical components of protein quality control pathways. These proteases have the responsibility of removing misfolded proteins that can occur during heat shock or stress. ClpAP is composed of a tetradecameric serine protease, ClpP (21.6 kDa monomer), and either the hexameric ClpA (84.2 kDa monomer) or ClpX (46.2 kDa monomer) ATPase/protein unfoldase. In addition to its proteolytic activity, ClpA has protein remodeling activity and therefore, in the absence of ClpP, is considered a molecular chaperone. From sequence analysis, ClpA has been found to be a member of the ATPases Associated with various Activities (AAA+) family of proteins. This family of proteins